The Mizo Academy of Sciences was established as the Mizo Post Graduate Science Society in 1995 and is chartered in Aizawl, India, as a non-profit, voluntary, scientific and educational organisation 'to promote and make further development of science for the Mizo community'. Since 2001 the society publishes *Science Vision*, with financial assistance from the Mizoram Council of Science, Technology & Environment, Directorate of Science & Technology.

Sci Vis is an interdisciplinary science journal, and is published quarterly in the months of March, June, September, and December. It aims to provide a forum for the reporting and discussion of news and issues concerning science, and to disseminate the information to the public. Though it is the official publication of the society, *Sci Vis* assumes no responsibility for opinions and conclusions drawn, or accuracy of data therein – including those of editorials, research articles, news, commentaries and reviews – which merely reflect the personal views of the authors and not official points of view of the MAS or the institutions/ organizations with which the authors are affiliated.

Sci Vis is indexed/listed in Google Scholar, Connect Journals, Index Copernicus, Ulrich's Periodical Directory, CiteSeerX, Open Academic Journals Index, Scientific Indexing Services, Scientific Journal Impact Factor, Genamics JournalSeek, and Citefactor.

Cover image: Chestnut-flanked white eye *Z. erythropleurus* at Murlen National Park [by Sailo *et al.*, see pp. 78-80]

SCIENCE VISION
ISSN (print) 0975-6175
ISSN (online) 2229-6026

CONTENTS

Volume 18, Number 2, April-June 2018

Instructions to Authors

Science Vision is a quarterly, refereed, and official journal of the Mizo Academy of Sciences. All kinds of information, both solicited and unsolicited, from all fields of science are published in the form of original research, research review, general article, hypothesis, commentary, report, letters and news. Manuscripts are accepted on the basis of credibility and suitability as per the style and scope of the journal.

Manuscript can be prepared in English (also in Mizo for general article). All texts should be in MS Word (.doc/docx), single spaced, and default font. There should not be any decorative borders, fonts, lines, indents and tabs anywhere. Tables and figures should come in separate pages at the end, and numbered consecutively as they are referred to in the text.

The title should be concise and plain, and the title page must contain full name(s) of the author(s), with complete mailing address of affiliation(s)/institute(s). In a multi-authored paper, the corresponding author should be marked with asterisk (*) after the name, and he/she must provide e-mail, and telephone/fax/mobile numbers.

Manuscripts containing original research and research review are subject to peer review. Original research can either be in full length or in short note articles. All original research should be necessarily written in English for the purpose of unanimity and universality.

The text for original research should be divided sequentially into **Title** page, **Abstract**, **Introduction**, **Materials and Methods**, **Results**, **Discussion**, and **References**. Abstract should be limited to 200-300 words for full length article, and to 100-200 for short article. Abstract should be accompanied by key words arranged in alphabetical order. Compliance to ethical standard for experiments involving animals or humans and clinical trials should be declared in the Materials and Methods.

References should be each numbered in consecutive Arabic numerals in superscripts in the text in the order of their appearance, and entry in the reference may follow the general APA style, or be in analogy to the examples:

1. **Journal**: Pachuau, Z., Zoliana, B., Zadeng, L. & Thapa R.K. (2003). Photoemission from surfaces of metals. *Science Vision* **3**, 17–19.
2. **Book/chapter:** Lalramnghinglova, H. (2003). State-of-the-art report on ethnomedicines and their plant resources in Mizoram. In: *Ethnomedicine of North-East India* (G. Singh, H.B. Singh & T.K. Mukherjee, eds), NISCAIR, CSIR, New Delhi, India, pp. 1–62.
3. **Internet:** Hopkin, H. (2004). Ancient ape gives clue to family origin. *news.nature.com/news* (18 November 2004).

Research review shall follow the organization as that of original research, excepting Materials and Methods and Results, which can be replaced with appropriate headings for the topic under discussion.

The manuscript should strictly adhere to internationally agreed rules of scientific nomenclature such as those adopted by the International Union of Pure and Applied Chemistry (IUPAC), the international system (SI) of units, and the International Code of Botanical/Zoological Nomenclature (ICBN/ICZN). Names of organisms should include the full binomial, authority, and family in the first reference in the body of the manuscript (e.g. *Homo sapiens* L.; family Hominidae).

Tables and figures should be placed after the main text (reference) starting in a anew page. Images should ne of high quality.

Submission is recommended through the e-mail: sciencevision@outlook.com. Submission will be taken to imply that it has not been published previously or concurrently submitted elsewhere, and that all authors have consented to its publication.

Manuscript not conforming to these instructions shall be summarily rejected.

To support an online open access, publication fee is levied as:

- ⊕ ₹1,000/- (US$50) for non-members.
- ⊕ ₹500/- (US$30) if the corresponding author is a member.
- ⊕ ₹250/- (US$10) if the corresponding author is a Life Member.
- ⊕ Additional ₹200/- (US$5) per page for colour.

Sci Vis **18 (2)**, 50—57 (2018)

SCIENCE VISION

Available at
www.sciencevision.org

Review Article

OPEN ACCESS

The saga of scrub typhus with a note on the outbreaks in Mizoram

K. Lalchhandama

Department of Zoology, Pachhunga University College, Aizawl 796001, India

Scrub typhus is one the most important re-emerging infectious disease, and perhaps, the most important bacterial disease. Caused by Orientia tsutsugamushi, it is transmitted through the bite of mites belonging to the genus Leptotrombidium, in which the bacteria are obligate parasites. Though the mites are natural ectoparasites of rodents and other animals, in which there is no disease, opportunistic infection to humans gives rise to a serious disease. Known to Japanese physicians as tsutsugamushi (insect disease), human infection is caused by the larvae of trombiculid mites, the fact established by Mataro Nagayo and co-workers established in 1917. The pathogen was discovered by Naosuke Hayashi in 1920. In Mizoram, the disease has been rampant since 2011. This paper summarises available data on the prevalence of the infection in different districts base on collective information from various sources. Records between 2012 and 2018 show that over a thousand people had been infected and 35 people had died of the disease.

Key words: Scrub typhus; *Orientia tsutsugamushi*; *Leptotrombidium*; rodent; Mizoram.

Received 06 February 2018
Accepted 09 March 2018

*For correspondence ✉:
chhandama@gmail.com

Contact us ✉:
sciencevision@outlook.com

Introduction

Scrub typhus has become arguably the most important bacterial disease in the world. It is caused by Gram-negative rickettsial bacteria called *Orientia tsutsugamushi*. Just like Augustus De Morgan rhyme, "Big fleas have little fleas upon their backs to bite 'em, And little fleas have lesser fleas, and so, *ad infinitum*," the bacteria are obligate parasites of trombiculid mites, which are in turn ectoparasites of animals such as birds, rodents and other mammals. In this ladder of life history, they are happy-going parasite with no ill intentions; but when infected mites bite accidental hosts such as humans, severe infections occur. They attack endothelial cells in the periphery as well as in the brain, but it also can be found in professional phagocytes such as macrophages of the liver and spleen.[1] As a consequence, clinical manifestations are presented by febrile fever, headache, myalgia, lymphadenopathy, and skin rash. In cases of misdiagnosis and failure of treatment, systemic complications rapidly develop including septic shock, acute respiratory distress syndrome, acute renal failure, meningitis, myocarditis, gastrointestinal bleeding, and multi-organ dysfunctions.[2,3]

The bacteria are oval shaped and measure 1.2 -3.0 μm in length and 0.5-0.8 μm in width. Adaptation to obligate parasitism resulted in reduced genome to about 2.4–2.7 Mb, which as it stands

is the most highly repeated bacterial genome sequenced. There are more than 30 antigenically different strains apart from the six important prototype serotypes – Gilliam, Karp, Kato, Shimokoshi, Kawasaki, and Kuroki.[4] They naturally infect trombiculid mites, of which *Leptotrombidium deliense* and *L. akamushi* are the two most important hosts. A continuum of transmission is maintained in the mite population, from adult to their eggs (transovarial transmission), and thence to larvae and adults (transstadial transmission).[1] The larvae, commonly referred to as chiggers, are the reservoirs and vectors as they are the only vertebrate-feeding stage. They normally feed on the extracellular body fluid of small mammals such as rodents; and wild rats of the species *Rattus* are the principal natural hosts. But the chiggers are opportunistic feeders and often attack birds and mammals, including humans.[5]

Clinical symptoms of scrub typhus are not specific, and are known to cause acute undifferentiated febrile illnesses, which are similar to those of malaria, leptospirosis, typhoid and dengue, making the infections difficult to diagnosed from the symptoms. For this confusion and difficulty in diagnosis, mortality at some point soared to 24%.[2] A dark, scab-like circle at the site of the chigger bite known as eschar is considered a valuable diagnostic clue,[6] but is not present or identifiable in most cases, and moreover, is by no means unique to scrub typhus – spotted fever and rickettsialpox also are indicated by eschar. Blood tests are also limited by non-specificity of the blood-bacteria (antibody-antigen) reaction, and sensitive immunological and molecular tests are expensive for mass diagnosis, particularly in endemic areas. For such problematic identification, the World Health Organization (WHO Recommended Surveillance Standards, 1999) had acknowledged that:

> "Scrub typhus is probably one of the most under-diagnosed and underreported febrile illnesses requiring hospitalization in the region. The absence of definitive signs and symptoms combined with a general dependence upon serological tests make the differentiation of scrub typhus from other common febrile diseases such as murine typhus, typhoid fever and leptospirosis quite difficult."[7]

Origin and Etymologies

Scrub typhus as a disease was known in 3rd century A.D. in China as indicated by legends and folktales. Japanese were more specific and familiar with the link between the infection and mites. They had given various names such as Nippon/Japanese river fever, akamushi (red mite) or kedani (hairy mite) disease of northern Japan, and most popularly as tsutsugamushi disease (Japanese *tsutsuga* means fever or harm or noxious, and *mushi* means bug or insect). Japanese physician Hakuju Hashimoto was the first to give a medical account of the disease from Niigita prefecture in 1810. He recorded the disease that he referred to as "*tsutsuga*" among the inhabitants of the banks of the upper tributaries of Shinano river.[8] The first report to the Western world was made by Theobald Adrian Palm, a physician of the Edinburgh Medical Missionary Society at Niigita, in 1878. He used the local name of the disease "*shima-mushi*", which he rendered in English as "island-insect disease".[9]

The name scrub typhus was given for the scrub vegetation of secondary growth in tropical regions as a result of clearing the primary forest where they (are believed to) originate. Coining the English name in 1929, William Fletcher, J. E. Lesslar and Raymond Lewthwaite at the Institute for Medical Research, Kuala Lumpur, F.M.S. Malaysia, wrote:

> The distribution of the K. form is very different from that of the W. form, which was later identified to be endemic typhus, it is essentially a disease of the open country and affects outdoor workers. It has a patchy distribution and outbreaks occur particularly in areas which, after being cleared of jungle, are allowed to grow up in weeds and scrub. For this reason, we propose the name scrub-typhus for the K. form of tropical typhus. Some of the cases of typhus-like diseases described in India are probably the same as

scrub-typhus.[10]

The agent of scrub typhus, *Orientia tsutsugamushi*, stands out among rickettisial or any other Gram-negative bacteria in having a cell wall that lacks lipophosphoglycan and peptidoglycan, which are otherwise hallmarks of bacteria. Its behavioural peculiarity caused much confusion as to its biological nature. It was first identified by Naosuke Hayashi at the Aichi Medical College, Nagoya, Japan, in 1920. Noticing peculiar "granular bodies" inside the red blood cells, lymphoid cells and in the blood plasma of infected patients, he concluded that these bodies were protozoans, the etiological agents of scrub typhus, remarking:

> I have reached the conclusion that the virus of the disease is the species of *Piroplasma* a protozoan in question. Among various species of *Piroplasma*, the cause of African cattle fever, *Thieleria parva*, (see Gonder, 1911), seems closely allied to the forms found in Tsutsugamushi disease, on account of morphological similarity, and of affinities for lymphocytes or endothelial cells... I consider the organism in Tsutsugamushi disease as a hitherto undescribed species, and at the suggestion of Dr. Henry B. Ward designate it as *Theileria tsutsugamushi*.[11]

Noting the major difference from protozoans, the name *Rickettsia orientalis* was introduced in 1930 by Mataro Nagayo and colleagues, who demonstrated the infection in the anterior chamber of the eye of the rabbit, and recognised the "virus" as member of the bacterial genus *Rickettsia*.[12,13] (*R. prowazekii* was the first rickettsial bacteria discovered, described by H. T. Ricketts and Russell M. Wilder in 1910. In 1916, Henrique da Rocha-Lima gave the name in honour of the pioneer Ricketts and his colleague Stanislaus Josef Mathias von Prowazek.[14,15]) Rinya Kawamüra and Yoso Imagawa inoculated the pathogen in the testicle of rabbits from which they confirmed the *Rickettsia*-like nature, and introduced the name *Rickettsia akamushi* in 1931. At the same time, Norio Ogata also gave the name *Rickettsia tsutsugamushi*, which he had identified in 1928 from experimental infection of rabbit testicle.[16] This name was accepted as a valid name for several decades. Akira Tamura and colleagues discovered novel properties of the scrub typhus bacterium such as thicker leaflet of the cell wall, peptidoglycan and lipopolysaccharide, such as muramic acid, glucosamine, hydroxy fatty acids, and 2-keto-3-deoxyoctonic acid. These features warrantee significant deviation from *Rickettsia* and designation of a novel genus. In 1995, they created *Orientia tsutsugamushi*,[17] crediting the geographical region and language of its original discovery.

The Mite Story

A systematic mite theory of the transmission of tsutsugamushi disease was formulated by Taichi Kitashima and Mikinosuke Miyajima in 1908.[18] In 1915, Hirst reported that the larval forms of trombiculid mites occurring in Japan are capable of transmitting Kedani or river fever. He reported the larvae of *Microtrombidium akamushi* present on the ears of field mice, thereby suggesting that mites carry and transmit the infection.[19] (French zoologist Émile Brumpt had described mite species in 1910 as *Trombidium akamushi* having seen only the larval specimens.) Back then the nature of the infectious agent was not yet resolved, and hence often referred to as either virus or protozoan of some sort. Miyajima and T. Okumura demonstrated the complete life cycle in 1917. They found that the larva is naturally attracted to field mice. They also experimentally infected Japanese monkey by letting the larval mites bite; and the monkeys developed severe symptoms of the infection. They named the mite as *Leptus Akamushi*.[18] In 1917, Mataro Nagayo and colleagues gave the first complete description of the developmental stages such as egg, nymph, larva, and adult; and noted that the larva is the carrier of tsutsugamushi disease in man. They observed that the nymph and adult do not bite humans or mammals.[20]

Hayashi successfully induced infection from human patients to guinea pigs in 1917. He observed granular bodies in the infected lymphoid

tissue.[21] That the larvae only feed they presume it was on blood once in each stage, and that adult also carried the "virus" indicating transtadial transmission in mites was reported by Nagay and co-workers in 1918.[22] Jūrō Hatori established in 1919 that the transmitter of the virus is a mite, apparently identical with the Japanese species, which he referref to as *Trombidiam* (*Trombicula*) *akamushi*. The natural hosts of this parasite include *Mus rattus rufescens* (common house rat of the island), *M. decamanus*, *M. musculus*,. agrarius, etc., and such insectivores as *Crocidura muschata*. He further established that mites acquire the virus in the adult stage and transfer it to their offspring and that the spread of the mites is chiefly due to the migration of their hosts, such as rodents, etc. But he failed to identify the pathogen.[23] By 1921, it was established that *T. akamushi* can parasitise a range of animals including, rats, mice, buffalos, dogs, cats, monkeys, and birds.[24] E. Walch described a new species of mite *Trombicula deliensis* in 1922 from Indonesia, as a vector for Japanese river fever.[25] In 1923, he demonstrated that out of several species of *Trombicula*, on three species, namely *T. pseudoakamushi*, *T. schüffneri* and *T. deliensis* do attack humans; and that *T. deliensis* was the principal carrier of the infection and that was found on rats.[26]

Rinya Kawamüra and Yoso Imagawa were the first to show that the *Rickettsia* "virus" are stored in the salivary glands of mites, and that mites feed on body (lymph) fluid as indicated by predilection of the pathogens in the lymph glands draining the area of the site of the primary sore or mite bite.[27] They were also the first to discover the *Rickettsia* bodies in the salivary glands of larval mites collected from infected field mice, thus establishing the facts beyond doubt that *Rickettsia* is the causative agent of tsutsugamushi and that mite larvae are the vectors.[28] Important discoveries made by Charles Nicolle (who won the Nobel Prize in Physiology or Medicine 1928 for his discovery of lice as the vectors of typhus) and his student Hélène Sparrow led to better understanding of the transmission of scrub typhus. In 1934, they reported that continuous infection could maintained from one rat to another, and symptoms never developed in rats; in contrast, inoculation in monkeys produced severe symptoms. The serum of some of the infected monkeys reacted to form agglutinins with *Proteus* OXK, but not OX19. Lice and fleas (*Xenopsylla cheopis*) fed on infected monkeys harboured viable "virus"; but only fleas could transmit during biting.[29]

Infestation in Mizoram

Now, I will try to make a collective report of scrub typhus cases in Mizoram through publicly available sources (**Table 1 and 2**). Fatal infections due to unspecified insect bite were known towards the end of the first millennium and beginning of the second millennium. The saga started with rumours and undescribed medical conditions. In 2011, senior physician Thangchungnunga wrote about deaths due to some unknown insect bite.[30,31] The same year, there was a clinical case at Champhai Hospital. A woman had severe attack of fever, and went to Champhai Hospital for diagnosis. The doctor there noted a dark scab on her body, but the doctor simply remarked that the birthmark – which most likely would have been an eschar – appeared to be growing, and dismissed the case as generic fever. Then, the fever struck with utmost severity and the patient fell unconscious. When she was taken to the MED-AIM Adventist Hospital, the vital signs were already gone. But with resuscitation, she was saved and survived the ordeal. As the case was discussed, it was retrospectively realised that at least 3 patients has similar conditions in the past, and at least 3 had died of similar conditions. It was then seriously suspected that scrub typhus was in Mizoram.[32]

The first medical records started in 2012 when the Health and Family Welfare Department, Government of Mizoram, launched the Integrated Disease Surveillance Programme. There is a vague reference of "Scrub typhus (The first clinical case report from Mizoram, India)" in 2011 at the 49th annual meeting of the Infectious Diseases Society of America (IDSA) in Boston, by George M. Varghese and Dilip Mathai

Table 1 | District-wise incidence of scrub typhus in Mizoram. Data compiled from various sources. Discrepancies (reports of IDSP are contradictory) are reconciled as far as possible.

District/Place	Incidence per year						
	2012	2013	2014	2015	2016	2017	2018
Aizawl East	98	28	85	35	0	81	0
West	112	54	49	23	0	0	0
Lunglei	0	47	38	3	0	0	162
Saiha	9	10	2	0	0	0	0
Champhai	20	26	4	0	41	0	0
Kolasib	6	0	0	0	0	0	0
Serchhip	4	3	0	0	0	0	0
Mamit	3	7	0	0	0	0	0
Lawngtlai	0	0	1	0	0	0	0
Referral Hospital	0	0	0	52	0	0	0
Total	252	175	179	113	41	81	162

Table 1 | Fatality cases due to scrub typhus in Mizoram compiled from various sources.

District/Place	Fatality cases per year						
	2012	2013	2014	2015	2016	2017	2018
Aizawl East	5	2	2	3	0	3	0
West	9	0	1	2	0	0	0
Lunglei	0	2	1	1	0	0	0
Saiha	0	0	0	0	0	0	0
Champhai	2	0	0	0	0	0	0
Kolasib	0	0	0	0	0	0	0
Serchhip	0	0	0	0	0	0	0
Mamit	0	0	0	0	0	1	0
Lawngtlai	0	0	0	0	0	0	0
Referral Hospital	0	0	0	1	0	0	0
Total	16	4	4	7	0	4	0

from the Christian Medical College, Vellore, Tamil Nadu. No such record is available with IDSA; although Varghese, Mathai and others did presented a paper "Scrub Typhus: Clinical and Laboratory Manifestations, Genetic Variability and Outcome" at CMC, but no mention of Mizoram.[33]) According to the 2017 official publication of the IDSP, there were 907 confirmed cases of scrub typhus in Mizoram between January 2012 and July 2017; with the total fatality amounting to 34.[34] Presenting slightly different epidemiology, IDSP entomologist Lalfakzuala Pautu stated that as of 2017, 985 people had scrub typhus, and 37 had died.[35] There were 252 cases in Mizoram in 2012. 16 of them died, out of which 14 were in Aizawl district and 2 were in Champhai district.[36]

In 2013, Vanlalrengpuia, Medical Officer at Khawbung, a village in Champhai district, encountered several febrile illnesses at the Primary Health Centre. He confirmed that 6 of them had scrub typhus.[37] It was then obvious that there was annual recurrence of the infection in Mizoram, and infection spread from one place to another. By 2015, record raised to 715 cases, with 31 fatality.[38] In 2016, there was an outbreak at Khawbung village in Champhai district, where 41 people were tested positive. Fortunately, all received medical treatment and survived.[39]

There was a huge commotion of infection in 2015 in Aizawl, the capital city of the state. Following the outbreak, 76 cases were examined at

Hunthar Veng in November. An investigative report of the IDSP Nodal Officer Lalmalsawma Pachuau declared that the it was not the case of scrub typhus, but a febrile inflammation due the bite of rove beetles, and that it was not lethal.[40] Although the false alarm was clinically proven, the media reports mentioned the causative as insect bite, which is factually not true. Rove beetles do not bite to induce inflammation. But they contain toxin called paederin. When they are accidentally brushed or crushed by humans, they excrete the toxin in their heamolymph. Paederin is an amide and is more potent than cobra venom.[41]

The first and only seroprevalence report was in 2017. The study conducted between October 2014 and November 2016 at Synod Hospital, Aizawl, revealed that out of 4081 human blood samples examined, 6.9% (n = 283) were positive in rapid-ICT test.[42]

Winter of 2017-2018 saw unprecedented outbreaks. Initial report indicated that outbreak started in December 2017 at Phullen village in Aizawl district, 47 people were infected claiming one person's life.[43] According to the official report on 5 January 2018, another 8 cases were diagnosed.[44] According to official IDSP news announcement, a total of 81 cases were rconfirmed at Phullen and surrounding villages such as Luangpawn, Thanglailung and Zawngin.[45,46]

Another wave of infection buffeted Lunglei district around the same time. Initial report claimed 33 cases with 3 mortalities at Haulawng village. Mass diagnosis by IDSP team confirmed additional 45 cases in January 2018.[47] According to Pachuau, infection was experienced in the village in early January. By the end of January, at least 161 people were infected at Haulawng and the neighbouring villages.[48] But according to Pautu, 162 cases were diagnosed in January 2018.[35]

Summing up the records, Lal Thanzara, Minister of Health and Family Welfare, reported at the Mizoram Legislative Assembly in March 2018 that 540 cases were confirmed during 2017-2018; 4 people died, 3 of them were from Aizawl district, and one from Mizoram-Tripura-Bangladesh border at Mamit district. He said that were found to be infected with the scrub typhus.[49] The infection was indiscriminate; R. Lalzirliana, Minister of Home Affairs, was diagnosed with scrub typhus at Aizawl Civil Hospital in January 2018.[50]

References

1. Chakraborty, S.; Sarma, N. (2017). Scrub typhus: An emerging threat. *Indian Journal of Dermatology*. **62** (5): 478–485.
2. Peter, J.V.; Sudarsan, T.I.; Prakash, J.A.J.; Varghese, G.M. (2015). Severe scrub typhus infection: Clinical features, diagnostic challenges and management. *World Journal of Critical Care Medicine*. **4** (3): 244–250.
3. Rajapakse, S.; Weeratunga, P.; Sivayoganathan, S.; Fernando, S.D. (2017). Clinical manifestations of scrub typhus. *Transactions of The Royal Society of Tropical Medicine and Hygiene*. **111** (2): 43–54.
4. Viswanathan, S.; Muthu, V.; Iqbal, N.; Remalayam, B.; George, T (2013). Scrub typhus meningitis in South India—a retrospective study. *PloS One*. **8** (6): e66595.
5. Luce-Fedrow, A.; Lehman, M.; Kelly, D.; Mullins, K.; Maina, A.; Stewart, R.; Ge, H.; John, H.; Jiang, J..; Richards, Allen (2018). A review of scrub typhus (*Orientia tsutsugamushi* and related organisms): then, now, and tomorrow. *Tropical Medicine and Infectious Disease*. **3** (1): 1–8.
6. Kundavaram, A.P.; Jonathan, A.J.; Nathaniel, S.D.; Varghese, G.M. (2013). Eschar in scrub typhus: a valuable clue to the diagnosis. *Journal of Postgraduate Medicine*. **59** (3): 177–178.
7. WHO (1999). *WHO Recommended Surveillance Standards* (Second edition). WHO/CDS/CSR/ISR/99.2. World Health Organization, Geneva, p. 124.
8. Kawamura, R. (1926). *Studies on tsutsugamushi disease (Japanese Blood Fever).* Cincinnati, OH (USA): Spokesman Printing Company. p. 2.
9. Palm, T.A. (1878). Some account of a disease called shima-mushi, or island-insect disease, by the natives of Japan; peculiar, it is believed, to that country, and hitherto not described. *Edinburgh Medical Journal*. **24** (2): 128–132.
10. Fletcher, W.; Lesslar, J.E.; Lewthwaite, R. (1929). The Aetiology of the Tsutsugamushi disease and tropical typhus in the Federated Malay States. Part II. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. **23** (1): 57–70.
11. Hayashi, N. (1920). Etiology of tsutsugamushi disease.

The Journal of Parasitology. **7** (2): 52–68.
12. Nagayo, M.; Tamiya, T.; Mitamura, T.; Sato, K. (1930). On the virus of tsutsugamushi disease and its demonstration by a new method. *Jikken Igaku Zasshi* (*Japanese Journal of Experimental Medicine*). **8** (4): 309–318.
13. Nagayo, M.; Tamiya, T.; Mitamura, T.; Sato, K. (1930). Sur le virus de la maladie de Tsutsugamushi [On the virus of tsutsugamushi]. *Compte rendu des seances de la Societe de biologie*. **104**: 637–641.
14. da Rocha-Lima, H. (1916). Zur aetiologie des fleckfiebers [The aetiology of typhus]. *Deutsche Medizinische Wochenschrift*. **42** (44): 1353–1354.
15. Arkwright, J.A. (1920). Remarks on the virus of typhus fever and the means by which it is conveyed. *Proceedings of the Royal Society of Medicine*. **13**: 87–95.
16. Ogata, N. (1931). Aetiologie der Tsutsugamushikrankheit: *Rickettsia tsutsugamushi* [Aetiology of the tsutsugamushi disease: *Rickettsia tsutsugamushi*]. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene*. **122**: 249–253.
17. Tamura, A.; Ohashi, N.; Urakami, H.; Miyamura, S. (1995). Classification of *Rickettsia tsutsugamushi* in a new genus, *Orientia* gen. nov., as *Orientia tsutsugamushi* comb. nov. International *Journal of Systematic Bacteriology*. **45** (3): 589–591.
18. Miyajima, M.; Okumura, T. (1917). On the life cycle of the Akamushi carrier of Nippon river fever. *Kitasato Archives of Experimental Medicine*. **1** (1): 1–14.
19. Hirst, S. (1915). On the Tsutsugamushi (*Microtrombidium akamushi*, Brumpt), carrier of Japanese river fever. *Journal of Economic Biology*. **10** (4): 79–82.
20. Nagayo, M. (1917). On the nymph and prosopon of the tsutsugamushi, *Leptotrombidium akamushi*, N. Sp. (*Trombidium akamushi* Brumpt), carrier of tsutsugamushi disease. *Journal of Experimental Medicine*. **25** (2): 255–272.
21. Hayashi, N. (1917). Studies on the pathogenesis of flood fever, tsutsugamushi. *Saikingaku Zasshi* (*Journal of Bacteriology*). **257**: 151–152.
22. Nagayo, M.; Miyagawa, Y.; Mitamura, T.; Tamiya, T. (1918). Ueber den nachweis des erregers der tsutsugamushi-krankheit im leibe des tsutsugamushi-muttertiers [On the Virus of Tsutsugamushi Disease in the Body of the Adult Mite]. *Verhandlungen der Japanischen Pathologischen Gesellschaft*. **8** (1): 142–144.
23. Hatori, J. (1919). On the endemic tsutsugamushi disease of Formosa. *Annals of Tropical Medicine and Parasitology*. **13** (3): 233–258.
24. Walch, E. (1922). Over *Trombicula deliensis*, n. sp. vermoedelijke over-brengster der Pseudotyphus, en andere Trombiculae van Deli [On *Trombicula deliensis*, n. sp., a Possible Transmitter of Pseudotyphus, and on the other Trombiculae of Deli. (First Communication.)]. *Geneeskundig Tijdschrift voor Nederlandsche-Indie*. **62** (5): 530–570.
25. Walch, E. (1923). On *Trombicula deliensis* probably carrier of the Pseudo-typhus and on other *Trombicula* species of Deli. *Kitasato Archives of Experimental Medicine*. **5** (3): 62–83.
26. Kawamuka, R.; Yamaguchi, M. (1921). Ueber die Tsutsugamushi-Krankheit in Formosa, zugleich eine vergleiehende Studie derselben mit der in Nordjapan [On the Tsutsugamushi Disease in Formosa, together witli a Comparative Study of the same in Northern Japan]. *Kitasato Archives of Experimental Medicine*. **4** (3): 169–206.
27. Kawamüra, R.; Imagawa, Y. (1931). Ueber die Proliferation der pathogenen *Rickettsia* im tierischen Organismus bei der Tsutsugamushi-Krankheit [The Multiplication of the Viras of Tsutsugamushi Disease *Rickettsia* in Animals]. *Transactions of the Japanese Society of Pathology*. **21**: 455–461.
28. Kawamüra, R.; Imagawa, Y. (1931). Die feststellung des erregers bei der tsutsugamushikrankheit [Confirmation of the infective agent in tsutsugamushi disease]. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene*. **122** (4/5): 253–261.
29. Nicolle, C.; Sparrow, H. (1934). Quelques expériences sur le virus de la fièvre fluviale du Japon (Tsutsugamushi). Compte *Rendu de l'Academie des Sciences*. **199** (24): 1349–1351.
30. Thangchungnunga (2011). Tunlaia Rannung Seh [Insect bites of these days]. *Dawrpui Veng Blog*.
31. Thangchungnunga (2012). Rannung Seh [Insect bite]. *Agape*. **25** (236). pp. 14–15.
32. Anon. (2011). Scrub typhus. *Champhai Kanan Veng*.
33. Varghese, G.; Prakash, J.; Abraham, O.; Mathews, P.; Thomas, K.; Mathai, D. (2011). Scrub Typhus: Clinical and Laboratory Manifestations, Genetic Variability and Outcome. 49th annual meeting of the Infectious Diseases Society of America (IDSA), Boston, MA, 21 October 2011.
34. Lalmalsawma, P.; Pautu, L.; Lalremsiama, N. (2017). Scenario of scrub typhus disease in Mizoram, Northeast India. *International Journal of Current Advanced Research*. **6** (10): 6341–6344.

35. Anon. (2018). Haulawngah rannung seh pung zel [Increasing insect disease at Haulawng]. *Zalen*.
36. Anon. (2013). Mizoramah rannung seh natna vangin Kum 4 chhungin mi 31 an thi tawh [31 deaths in 4 years due to insect disease in Mizoram]. *Vanglaini*.
37. Anon. (2015). Rannung seh vangin mi 3 damdawi-inah [3 hospitalised due to insect bite]. *Vanglaini*.
38. Press Trust of India (2015). 31 typhus deaths in Mizoram in 4 yrs. *India Today*.
39. Anon (2016). Disease alerts/outbreaks reported and responded to by states/UTs through Integrated Disease Surveillance Programme (IDSP) (PDF). Integrated Disease Surveillance Programme (IDSP).
40. Anon. (2015). Tunlaia rannung seh a hlauhawm loh [Present insect bites are not dangerous]. *Vanglaini*.
41. Nasir, S.; Akram, W.; Khan, R.R.; Arshad, M.; Nasir, I. (2015). *Paederus* beetles: the agent of human dermatitis. *The Journal of Venomous Animals and toxins including tropical diseases*. **21**: 5.
42. Lalrinkima, H.; Lalremruata, R.; Lalchhandama, C.; Khiangte, L.; Siamthara, F.H.; Lalnunpuia, C.; Borthakur, S.K.; Patra, G. (2017). Scrub typhus in Mizoram, India. *Journal of Vector Borne Diseases*. **54** (4): 369–371.
43. Press Trust of India (2018). Scrub typhus virus: One person dies, 47 infected in Mizoram. *The New Indian Express*.
44. Anon. (2018). Phullen khua a scrub typhus leng en fiaha kalte report [Report of the scrub typhus investigation team at Phullen village]. Health & Family Welfare Department, Government of Mizoram.
45. PTI (2018). Scrub typhus virus: One person dies, 47 infected in Mizoram. *The New Indian Express*.
46. Media Scanning & Verification Cell (2018). Mizoram: Scrub typhus infection reported in Lunglei district, Mizoram (PDF). Integrated Disease Surveillance Programme (IDSP), National Centre for Disease Control, Ministry of Health & Family Welfare, Government of India.
47. Anon. (2018). Haulawng khuaah Scrub Typhus a leng [Outbreak of scrub typhus at Haulawng]. *Vanglaini*.
48. Anon. (2018). Scrub Typhus Cases Rise to 161 in Mizoram. *Northeast Today*.
49. Press Trust of India (2018). Four die in scrub typhus infection in Mizoram. *Business Standard*.
50. Hmar, S. (2018). Mizoram home minister down with Scrub Typhus, disease not contained. *Northeast Now*.

Sci Vis 18 (2), 58—63 (2018)

SCIENCE VISION

Available at
www.sciencevision.org

Research Article

OPEN ACCESS

Comparison of single and double entry twin cup dosimeter in measuring indoor radon and thoron concentration in Mizoram, India

L.Z. Chhangte[1], P.C. Rohmingliana[2], B.K. Sahoo[3], B.K. Sapra[3], Vanramlawma[1], Hmingchungnunga[1], Remlalsiama[1], Z. Pachuau[1], B. Zoliana[2*]

[1] Department of Physics, Mizoram University, Tanhril, Aizawl 796004, India
[2] Department of Electronics, Zirtiri Residential Science College, Aizawl 796001, India
[3] Radiological Physics and Advisory Division, Bhabha Atomic Research Centre, Mumbai 400085, India

Indoor radon and thoron concentrations have been measured using solid state nuclear track detector (LR-115 type-II) based twin cup dosimeter with single and double entry deployed side by side. The measurements have been carried out in 50 dwellings of 8 different villages/ towns situated in Saiha and Lawngtlai districts, Mizoram. Dwellings were selected primarily from the gamma level measured using Micro-R survey meter and the construction type of the building. The average concentrations of radon and thoron were found to be 75.76 Bq/m^3 and 96.50 Bq/m^3 for single entry dosimeter and 63.47 Bq/m^3 and 19.79 Bq/m^3 for double entry dosimeter. The single entry dosimeter was found to give more reliable observations than that of double entry in terms of trends of seasonal data and also on the theory behind the manufacturing qualities.

Keywords: Twin cup dosimeters, radon, thoron, solid state nuclear track detectors, gamma level.

Received 15 December 2017
Accepted 26 February 2018

*For correspondence ✉:
bzoliana@gmail.com

Contact us ✉:
sciencevision@outlook.com

Introduction

Radon (^{222}Rn) is a natural radioactive gas, which is produced from radium (^{224}Ra) and which in turn is a decay product of uranium (^{238}U), is continually formed in soil and released to the air through the processes called emanation and exhalation.[1-5] It is now widely understood that the most important component of radiation exposure to the public is due to the inhalation of indoor radon, thoron and their decay products. Even more significant is that the estimated level of health risk associated with average indoor radon levels is much higher than those due to other environmental carcinogens. Monitoring in various countries[6] yields average residential ^{222}Rn concentrations ranging from 10 to 100 Bq/m^3. The indoor concentration and its decay products depends on three factors; the entry or production rate from various sources, the ventilation rate and the rates of chemical or physical transformation or removal.

The main sources of indoor radon, thoron and their decay products are the soil-gas, minerals and rocks in the earth's crust, building material and ground water. Type of houses, ground

soil, ventilation rate, atmospheric pressure and temperature affects the equilibrium factor,[7] which also has a significant role in indoor gas measurements. Radon's daughter elements can be hazardous than radon itself unless it is concentrated in an enclosed space.[8] As radon gas is inhaled, densely ionizing alpha particles emitted by the short-lived (3.82 days) decay products of radon (^{212}Po and ^{214}Po) can interact with biological tissue in the lungs leading to DNA damage and results into lung cancer.[9] The world average of annual effective dose to the human by natural radiation is 2.4 mSv/y and about half of which is due to internal exposure to progenies of radon and thoron.[10] Thus, it is of fundamental importance to measure their concentration correctly.

In the present study, passive measurement technique of radon and thoron has been followed, using solid state nuclear track detector (SSNTD) LR-115, type-II based twin cup dosimeter with single[11] and double entry.[12-13] These dosimeters were developed in Bhabha Atomic Research Centre (BARC), Mumbai.

Materials and Methods

Dosimeters

The twin cup dosimeter with double entry consists of a cylindrical plastic chamber divided into two equal compartments, each having a length of 4.1 cm and height 3.1 cm. The LR-115 type II films are then inserted at the compartments. The SSNTD placed in one compartment measures radon alone which diffuses into it from the ambient air through a semi-permeable membrane of 25 µm thickness. It allows the build-up of about 90% of the radon gas in the compartment and suppresses thoron gas concentration by more than 99%. On the other hand, the glass fiber filter paper of thickness 0.56 mm in the other compartment allows both radon and thoron gases to diffuse in and hence the tracks on SSNTD placed in this chamber are related to the concentrations of both the gases. However, the estimation of the gas concentrations using this dosimeter was based on the assumption of the same entry rate of the gases into the two cups of the dosimeter, which may not be valid for dosimeters deployed in turbulent environmental conditions. To overcome this limitation, a new pin-hole based ^{222}Rn/^{220}Rn discriminating measurement device has been developed (Fig. 1).

The new design of this dosimeter system has two compartments separated by a central pinholes disc made up of High Density Polyethylene (HDPE) material, acting as ^{220}Rn discriminator. Each chamber has a length of 4.1 cm and radius of 3.1 cm (same dimensions as in the twin cup dosimeter.[14] The first compartment (named as 'radon + thoron' chamber) samples ambient air

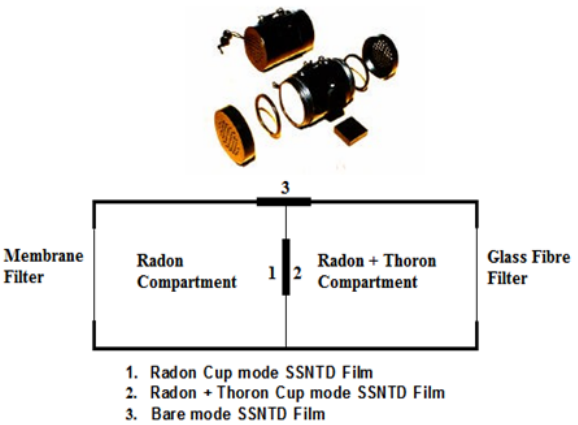

Figure 1 | Double entry dosimeter.

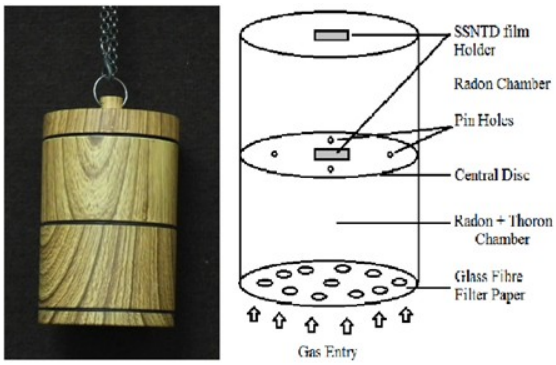

Figure 2 | Single entry dosimeter.

at the entry of which, the particulates are restricted by using a filter paper. The air containing radon and thoron from this compartment diffuses to the second compartment (named as "radon" chamber) through four pin-holes of 0.5 mm radius, acting as a diffusion barrier that cuts off the entry of thoron into this chamber due to its short half-life (55.6 s). Hence, only radon enters into this compartment. The tracks registered in LR-115 placed in first and second chamber are corresponding to the radon + thoron and radon concentration in the atmosphere respectively.

Salient features of the device include (i) the pinholes act as $^{222}Rn/^{220}Rn$ discriminator and eliminate the requirement of membrane filter used in the earlier twin cup design (ii) the single entrance design for gas transmission and (iii) use of multiple pinholes of reasonably small radius minimises effect of turbulence on $^{222}Rn/^{220}Rn$ transmission factors so that the calibration factor is independent of indoor turbulence.

Measurements

The two types of dosimeter with LR-115 type-II plastic track detectors (SSNTDs) kept inside them was deployed side by side in 50 dwellings of 8 different villages of Lawngtlai and Saiha districts, Mizoram India from 10 January 2017 to 10 May 2017. Due to maximum occupancy, bed rooms were chosen for deployment of the dosimeters. The GPS co-ordinates have also been recorded for each dwelling in which dosimeters were installed for proper location assessments. The dwellings in each village were selected on the basis of gamma readings as well as construction type of the buildings.[15]

After four months of exposure in dwellings, the detectors were removed and subjected to chemical etching in 2.5 N NaOH solutions at 60°C for 90 minutes in a constant temperature bath. Then these films were washed and dried. The track density was obtained by using the standard spark counter (Model PSI-SC1) with operating voltage (500 V) and the pre-sparking voltage (900 V) of the spark counter, which has been established before these measurements.[16]

Calculation

Formula used for calculating radon concentration from the track density of radon chamber of the dosimeter.

$$C_R(Bq/m^3) = \frac{T_R}{CF \times T}$$

where C_R is the radon concentration and T_R is the tract density of films in radon chamber. T is the exposure period in days. Calibration factor[11] (CF) used are 0.0172 ± 0.002 for single entry dosimeter and 0.021 ± 0.0004 for dual entry dosimeter[14].

Formula used for calculating thoron concentration from the track density of radon + thoron chamber of the dosimeter.

$$C_T(Bq/m^3) = \frac{T_F - T_P}{CF \times T}$$

where C_T is the thoron concentration, T_F is the track density of films in radon chamber and T_P is that for radon + thoron chamber. T is the exposure period in days. Calibration factor[11] (CF) used are 0.010 ± 0.001 for single entry dosimeter and 0.019 ± 0.002 for dual entry dosimeter.[14]

Result and Discussion

Figure 3 show the comparison graph for the concentration of indoor radon measured with single and double entry twin cup dosimeters. The radon concentration measured with the single-entry dosimeter range from 27.09-206.29 Bq/m^3 with the average value of 75.76 Bq/m^3, GM of 69.58 Bq/m^3, SD of 34.67 and GSD of 1.49, whereas double entry dosimeter registers the concentration range of 32.42-109.06 Bq/m^3 with the average value 63.47 Bq/m^3, GM of 61.52 Bq/m^3, SD of 16.21 and GSD of 1.28. The single-entry dosimeter registers higher concentration value in 60% of the dwellings where the two types of dosimeter are deployed.

Figure 4 shows the comparison graph for the concentration of indoor thoron measured with single and double entry twin cup dosimeters. The thoron concentration measured with the

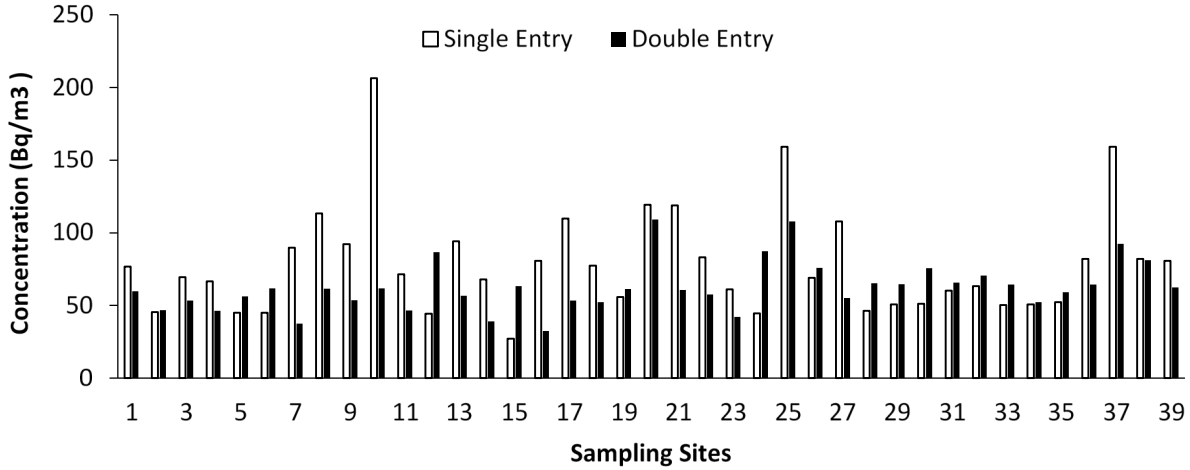

Figure 3 | Radon concentration from single and double entry dosimeter.

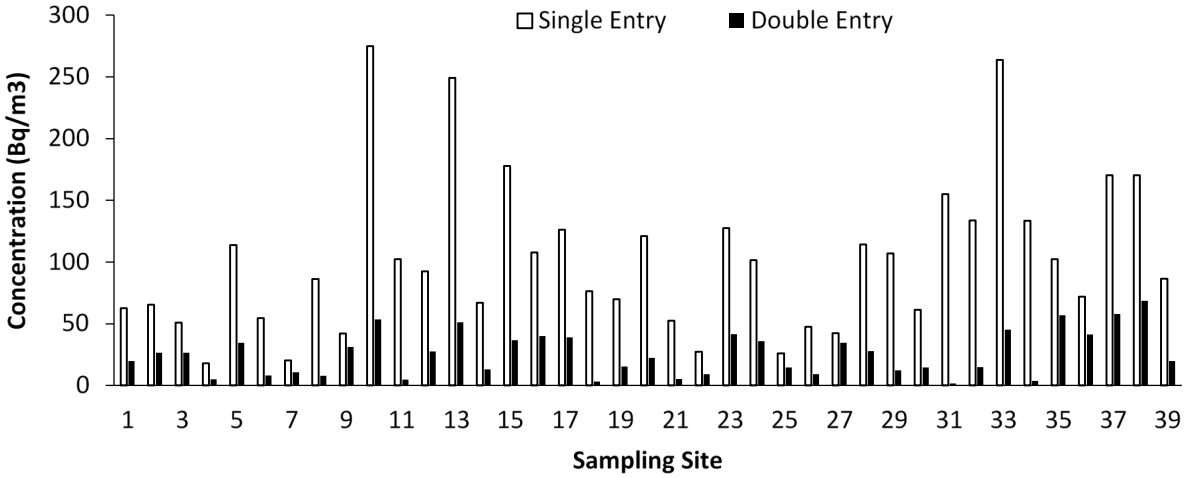

Figure 4 | Thoron concentration from single and double entry dosimeter.

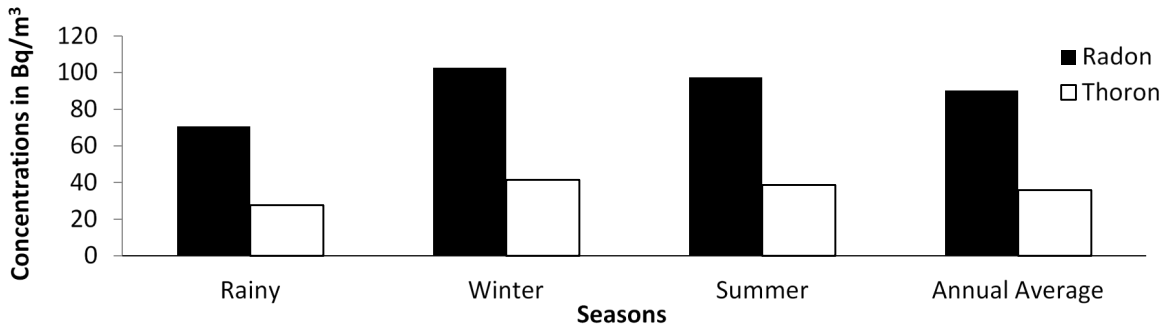

Figure 5 | Seasonal variations of radon/thoron using single entry twin cup dosimeter.

single-entry dosimeter range from 17.98-177.97 Bq/m^3 with the average value of 89.34 Bq/m^3, GM of 76.93 Bq/m^3, SD of 44.31 and GSD of 1.81 whereas double entry dosimeter registers the concentration range of 1.78-68.69 Bq/m^3 with the average value 23.48 Bq/m^3, GM of 16.88 Bq/m^3, SD of 17.18 and GSD of 1.68. The single-entry dosimeter registers higher concentration value in all of the dwellings where the two types of dosimeter are deployed. The high value of radon and thoron concentration registered are all in full concrete type of building with one of the walls adjacent to the soil.

Our result shows that indoor concentration of radon and thoron register by the single-entry dosimeter is comparatively higher than those of the double entry dosimeter. At the same time, the size and dimension of pin-hole in single cup dosimeter were exactly as specified. However, the manufacturer's defect was seen in some of the double entry twin cup dosimeters where the size of pin hole was not regular, but tapering at the end. The error made with the assumption of equal entry of the gas in both sides of the double entry dosimeter and the ventilation system of dwellings may not play much role in this the gas concentration while the dosimeters are deployed.

Besides, it is also found that single entry dosimeters data[17] obeys the trend that is expected for seasonal concentration of the gases (Fig. 5) *viz.* it is highest in winter followed by summer and lowest in rainy season where we do not have such observation in previous data.[18]

Thus, we have concluded that for passive long time integrated method of measurement, the use of single-entry cup dosimeter may be more reliable than that of double entry dosimeter.

Acknowledgement

The authors are grateful to Radiological Physics and Advisory Division, Bhabha Atomic Research Center for providing necessary equipment and all the resident in the study area who have helped in carrying out this study.

References

1. Menon, M.R., Lalith,B .Y. &Shukla, V.K. (1987). Natural radioactivity in building materials in India. *Bullettin of Radiation Protection* **14**, 45–48.
2. Shukla, V.K., Ramachandran, T.V., Chinnasakki, S., Sartendal, S.J. & Shanbhag, A.A. (2005). Radiological impact of utilization of phophogypsum and fly ash in building materials in India. *International Science Congress Series* **1276**, 339–340.
3. Sahoo, B.K., Nathwani, D., Eappen, K, P., Ramachandran, T.V., Gaware, J.J. & Mayya, Y.S. (2007). Estimation of radon emanation factor in indian building materials. *Radiation Measurements* **42**, 1422–1425.
4. Sahoo, B.K. (2008). Theory of radon emanation and method of source term estimation by flux measurement technique. *Proceedings of DAE – BRNS Theme Meeting, Radon – 2008*, 18–24, March 11–13, 2008.
5. Sahoo, B.K. & Mayya, Y.S. (2010). Two-dimensional diffusion theory of trace gas emission into soil chambers for flux measurements. *Agriculture and Forest Meteorology* **150**, 1211–1224.
6. Nazaroff, W.W. & Nero, A.V. (1988). *Radon and its Decay Products in Indoor Air*. John Wiley & Sons, New York.
7. Abu-Jarad, F. & Fremlin, J.H. (1983). Effect of internal wall covers on radon emanation inside houses. *Health Physics Journal* **44**(3), 243–248.
8. Majumdar, D. (2000). Radon in the environment and associated health problems. *Resonance* July, 44–55.
9. Edling, C., Wingreen, G., & Axelson, O. (1986). Quantification of the lung cancer risk from radon daughter exposure in dwellings - An epidemiological approach. *Environment International* **12**(1–4), 55–60.
10. UNSCEAR. *United Nations Scientific Committee on the effects of Atomic Radiation*. Sources, effects and risks of ionizing radiation, United Nations 2000.
11. Sahoo, B.K., Sapra, B.K., Kanse, S.D, Gaware, J.J & Mayya Y.S. (2013). A new pin-hole discriminated ^{222}Rn/^{220}Rn passive measurement device with single entry face. *Radiation Measurements* **58**, 52–60.
12. Mayya, Y.S., Eappen, K.P. & Nambi, K. S.V. (1998). Methodology for mixed field inhalation dosimetry in monazite areas using a twin cup dosimeter with three track detectors. *Radiation Protection Dosimetry* **77**, 177–184.
13. Nambi, K.S.V., Ramu, M.C.S., Eappen, K.P.,

Ramachandran, T.V., Muraleesharan, T.S. & Shaikh, A.N. (1994). A new SSNTD method of combined measurement of radon and thoron working levels in atmosphere. *Bulletin of Radiation Protection* **17**, 34–35.

14. Eappen, K.P. & Mayya, Y.S. (2004). Calibration factors for LR-115 (Type-II) based radon thoron discriminating dosimeter. *Radiation Measurements* **38**, 5–17.
15. Vanchhawng, L. (2012). *Measurement of radon, thoron and their progeny concentrations in Mizoram with special reference to Aizawl, Champhai and Kolasib Districts.* Unpublished PhD thesis at Mizoram University.
16. Azimi-Garakani, D., Shahbazi, M. & Latifi, G. (1981). A new automatic spark counting system. *Nuclear Tracks* **4**, 141–148.
17. Chhangte, L.Z., Rohmingliana, P.C., Sahoo, B.K., Sapra, B.K., Zoliana, B. & Pachuau, Z. (2017). Seasonal variation of indoor radon and thoron concentration in Mizoram, India. Presented at *20th National Conference on Solid State Nuclear Track Detectors and their Application* at Vidya Vikas Institute of Engineering and Technology, Mysuru on 26-28, October, 2017.
18. Rohmingliana, P.C., Lalmuanpuia. V., Thapa, R.K., Sahoo, B.K., Mishra, R, Mayya, Y.S. & Zoliana, B. (2011). Seasonal variation of radon/thoron and their progeny concentration in Saiha District, Mizoram, India. *Advances in Environmental Chemistry* **15**, 193–195.

Sci Vis 18 (2), 64—69 (2018)

SCIENCE VISION

Available at
www.sciencevision.org

Research Article

OPEN ACCESS

Estimation of cholesterol in different edible oils found in Mizoram

Lalnunhruaitluangi*, Amit Powdel, K. Vanlalzarliani, David Lalnunzira, Susankar Kushari, Suman Kumar, Saddique Tapadar, Lalzikpuii Sailo

Department of Pharmacy, Regional Institute of Paramedical and Nursing Sciences, Aizawl 796017, India

The study was performed to investigate the quantity of cholesterol level in the commonly consumed fats and oils in Mizoram, and further assessed, whether or not it is of risk to coronary heart diseases (CHD). Samples collected include mustard oil (Vimal), butter (Amul), dalda (Vanaspati), ghee (Amul and Vanaspati), refined oil (Best Choice, Dhara, Nutrela, and Gokul), coconut oil (Parachute), olive oil and palm oil. Performing the experiment gives a result where palm oil was found to contain highest level i.e. 804.5 mg/L and coconut oil (Parachute) has the lowest i.e. 179 mg/L. Daily requirements of cholesterol is estimated to be approximately 300 mg per day and thus showing the tested samples to be safe for human consumptions but however limit use of edible oils is recommended for safety measures for high risk individual.

Key words: Acid value, cholesterol, coronary heart disease (CHD), Liebermann-Burchard method, peroxide value, saponification value.

Received 08 March 2018
Accepted 27 April 2018

*For correspondence ✉:
hruaiifanaitorel@gmail.com

Contact us ✉:
sciencevision@outlook.com

Introduction

Cholesterol from food is hard to get away from, even though one may be watching his/her diet. All foods of animal origin contain cholesterol, including eggs, red meat, and shrimp. Generally, foods that are high in saturated fats or trans fats should also be limited. These include foods you may not even think of, such as grilled-cheese sandwich, margarine, potato with butter and chicken pot pie. As we eat, cholesterol from food is absorbed by our digestive tract. It then makes its way into our liver and can circulate through our body in the bloodstream.[1] Cholesterol, a lipid plays a vital role in the physiological regulations of membrane fluidity and proper functioning of cells. It is also a major precursors in the production of bile acids, steroids hormones as well as vitamin D. Cholesterol found in the cell membranes of the cells, has been of great medical importance in recent years because its high level in the body has been associated with coronary heart diseases. Coronary heart disease (CHD) is a leading cause of death in most industrialized countries and its importance as a major health problem is increasing in developing countries.[2]

Keeping total fat intake low is an important way to lower cholesterol and reduce the risk of other chronic diseases. Animal products, includ-

ing meat and dairy products, as well as fried food and vegetable oils are all loaded with fat. The most important piece of information to look for is the percentage of calories from fat.[3] Therefore, to check the percentage of calories of fats, edible oils as being the common source are taken into consideration. Industrial processing especially catalytic hydrogenation of vegetable oils affects their fatty acid composition Processing increases saturated fatty acid component of oils. Saturated fatty acid rich diets have been found to increase the level of cholesterol.[4]

But what is often not realized is that a much bigger source of cholesterol is body itself. About 1 g of cholesterol is daily synthesized in the body and all the 27 carbon atoms of cholesterol are synthesized from acetyl-COA.[5] Intake of certain fats such as saturated fats, which are present in animal fat and which solidify in winter, such as coconut oil, palm oil and hydrogenated oils (Vanaspati) raise the cholesterol level. On the other hand, taking vegetable oils known as poly-unsaturated fats like safflower oil, mustard oil and sunflower oil lower the cholesterol. This is also done by taking mono-unsaturated fats like olive oil.[6]

Materials and Method

Sources of edible vegetable oils sold in market of Aizawl include mustard oil (Vimal), butter (Amul), dalda (Vanaspati), ghee (Amul and Vanaspati), refined oil (Best Choice, Dhara, Nutrela, and Gokul), coconut oil (Parachute), olive oil and palm oil. Samples of ten brands of edible oils were collected. Samples of two non-branded vegetable oils are were purchased from Aizawl in which they are produced in small scale.

Cholesterol estimation

Cholesterol content was estimated using Liebermann-Burchard reagent.[7] Standard cholesterol solution used was 2 mg/ml as stock solution. Liebermann-Burchard reagent was prepared with 7 ml concentrated sulfuric acid and 5 ml glacial acetic acid and was covered with black paper and kept in ice bucket in dark place.

Six volumetric flasks were marked as s1, s2, s3, s4, s5 and s6. Standard cholesterol solution was added as 0.4, 0.6, 0.8, 1.0 and 1.2 ml in five volumetric flasks whereas, flask six was kept blank. Two ml of the Liebermann-Burchard reagent were added to all six volumetric flasks and diluted to final volume of 10 ml with chloroform (**Table 1**). Flasks were covered with black carbon paper and kept in dark for 15 min. Then, set zero of spectrophotometer with blank (s6) at 640 nm. The absorbance of all standards (six flasks) were determined on UV/Vis spectrophotometer (Thermo Scientific, Empower software) and standard graph was plotted (**Figure 1**). Three ml of sample solutions were taken and their absorbance were determined on UV/Vis spectrophotometer after adding 1 ml oil sample, 2 ml Liebermann-Burchard reagent and 7 ml chloroform. Cholesterol concentration of sample solu-

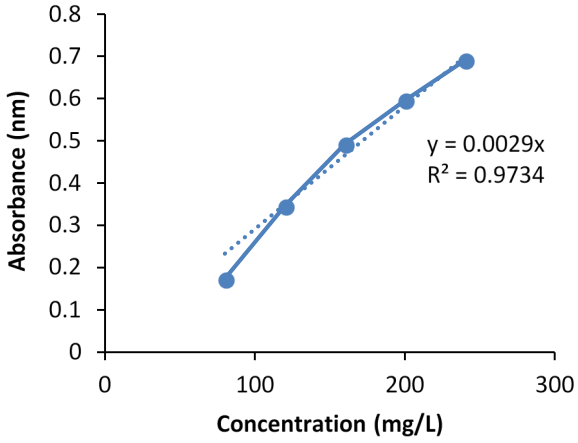

Figure 1 | Standard curve of cholesterol.

Table 1 | Lieberman-burchard method for cholesterol estimation.

Reagents (ml)	S1	S2	S3	S4	S5	S6
Standard cholesterol solution	0.5	1	1.5	2	2.5	-
Lieberman-Burchard reagent	2	2	2	2	2	2
Chloroform	4.5	4	3.5	3	2.5	5

Table 2 | Cholesterol content of the samples. Data analyzed by one-way ANOVA followed by Tukey-Kramer test for n=3.

Sl no	Samples	Mean±SEM	Cholesterol content (mg/L)
1	Best Choice refined soya bean oil	0.685±0.264	342.5
2	Dhara refined soya bean oil	0.804±0.146	420
3	Nutrela refined soya bean oil	0.949±0.050	474.5
4	Gokul soya bean oil	0.628±0.235	314
5	Vimal mustard oil	0.818±0.112	409
6	Olive oil	1.436±1.150	718
7	Parachute coconut oil	0.358±0.198	179
8	Palm oil	1.609±0.314	804.5
9	Amul butter	0.525±0.152	262.5
10	Amul ghee	0.422±0.221	211
11	Vanaspati ghee	1.074±0.879	537
12	Vanaspati dalda	0.440±0.305	220

tions was determined (Table 2) using a standard curve constructed graphically plotting the absorbance against mg/l cholesterol.

Acid value

Each oil sample (1.0 g) was weighed and dissolved with 50 ml of ethanol in a conical flask. Two drops of phenolphthalein indicator were added and titrated to pink end point (which persisted for 15 minutes) with 0.1 N potassium hydroxide solution (KOH). Acid value was calculated by the equation:

$$\text{Acid value} = \frac{56.1 \times V \times C}{M}$$

Where 56.1 is equivalent weight of KOH, V is the volume in ml of standard volumetric KOH solution used, C is the exact concentration in KOH solution used (0.1 N); m is the mass in grams of the test portion (1 g).[2]

Saponification value

Saponification value was determined according to titremetric method.[8] 2 grams of oil samples were weighed into a conical flask and 25 ml ethanolic potassium hydroxide was added. The solution was refluxed for 2 h with time to time shaking. 1 ml phenolphthalein was added and titrated with 0.5 N hydrochloric acid (HCl). The same process was conducted for blank determination. The value was calculated as follows:

$$\text{Saponification value} = \frac{56.1 \times (V_0 - V_1) \times C}{M}$$

Where 56.1 is equivalent weight of KOH, V_0 is the volume in ml of standard HCl solution used for the blank test, V_1 is the volume in ml of the standard HCl solution used for sample, C is the exact concentration of the standard HCl (0.5 N) solution and m is the mass in gram of the test portion (2 g).

Peroxide value

Peroxide value was evaluated according to AOCS official method.[9] 5 grams oil samples were weighed into a conical flask and 30 ml of solvent mixture of glacial acetic acid-chloroform in the ratio of 3:2, respectively, were added to the oil samples. Half ml saturated potassium iodide (KI) solution was added to the solution and allowed to stand for 1 min thereafter, 30 ml of distilled water were added and titrated with 0.01 N sodium thiosulfate solution using starch indicator

until the yellow color was discharged. A blank was prepared alongside the oil samples. Peroxide value was calculated.

$$\text{Peroxide value} = \frac{10 \times (V_1 - V_2)}{M}$$

Where V_1 is the volume of $Na_2S_2O_3$ for determination of test sample in ml, V_2 volume of $Na_2S_2O_3$ for determination of blank solution in ml, and m is mass of test portion in g (5 g).

Results

Experiments were performed by analyzing the samples for their cholesterol content. Apart from this, acid value, saponification value and peroxide value were also estimated.

The cholesterol content was estimated by Lieberman-Burchard test. The results (**Table 2**) showed that palm oil contains the highest (804.5 mg/L) among the tested samples and the lowest being Parachute coconut oil (179 mg/L). The study is useful to predict whether the edible oils used for cooking are responsible for cardiovascular related diseases. However, the study proved the safety of the tested samples.

Acid value is a measure of the free fatty acids in oil. Normally, fatty acids are found in the triglycerides form, however, during processing the fatty acids may get hydrolyzed into free fatty acids. The higher the acid value found, the higher the value of the fatty acids which translates into decreased oil quality. The study (**Table 3**) showed that Palm oil (2.7803 mg KOH/g) have the highest acid value followed by Vimal mustard oil (2.3090 mg KOH/g) and Gokul soya bean oil (2.1273 mg KOH/g) and the lowest being Best Choice refined soya bean oil (0.9828 mg KOH/g).

Saponification value is an indication of the molecular weights of triglycerides in oil and indicates high proportion of lower fatty acids since it is inversely proportional to the average molecular weights or chain length of the fatty acids. Therefore, shorter the average chain length, the higher is the saponification number. **Table 4** showed that Vanaspati Dalda has significantly lowest saponification value (108.974 mg KOH/g) and hence are not suitable for human nutrition.

Peroxide value is a measure of oxidation during storage and the freshness of lipid matrix. In addition, it is a useful indicator of the early stages of rancidity occurring under mild condition and it is a measure of primary lipid oxidation products. One of the most important parameters that influence lipid oxidation is the degree of unsaturation of its fatty acids. When double bonds of unsaturated fats are oxidized, peroxides are among the oxidation products formed. High peroxide value is an indicator of

Table 3 | Acid value of the samples. Data analyzed by one-way ANOVA followed by Tukey-Kramer test for n=3.

Sl no	Samples	Mean±SEM	Acid Value (mg KOH/g)
1	Best Choice refined soya bean oil	1.46±0.05	0.9828
2	Dhara refined soya bean oil	1.8±1.327	1.2117
3	Nutrela refined soya bean oil	1.6±0.10	1.0771
4	Gokul soya bean oil	3.16±0.057***	2.1273
5	Vimal mustard oil	3.43±0.577***	2.3090
6	Olive oil	1.83±0.10	1.2319
7	Parachute coconut oil	1.66±0.057	1.1175
8	Palm oil	4.13±0.57***	2.7803
9	Amul butter	2.03±0.05	1.3665
10	Amul ghee	1.5±0.173	1.0098
11	Vanaspati ghee	1.73±0.28	1.1646
12	Vanaspati dalda	2.1±0.17	1.4137

***P<0.001, **P<0.01, *P<0.05.

Table 4 | Saponification value of the samples. Data analyzed by one-way ANOVA followed by Tukey-Kramer test for n=3.

Sl no	Samples	Mean±SEM	Acid saponification value (mg KOH/g)
1	Best Choice refined soya bean oil	46±1.00	126.225
2	Dhara refined soya bean oil	46.5±0.30	119.212
3	Nutrela refined soya bean oil	45.9±0.10	127.627
4	Gokul soya bean oil	45.73±0.25	130.012
5	Vimal mustard oil	46.03±0.35	125.805
6	Olive oil	46.4±0.40	120.615
7	Parachute coconut oil	41.83±0.76	184.709
8	Palm oil	42.4±0.80	176.715
9	Amul butter	38.4±5.40	232.815
10	Amul ghee	43.6±0.36	159.885
11	Vanaspati ghee	43.93±0.90	155.256
12	Vanaspati dalda	47.23±0.25*	108.974

***P<0.001, **P<0.01, *P<0.05.

Table 5 | Peroxide value of the samples. Data analyzed by one-way ANOVA followed by Tukey-Kramer test for n=3.

Sl no	Samples	Mean±SEM	Peroxide value (meg peroxide/g)
1	Best Choice refined soya bean oil	6.76±0.145***	9.52
2	Dhara refined soya bean oil	7.93±0.348***	11.86
3	Nutrela refined soya bean oil	11.56±0.284***	19.12
4	Gokul soya bean oil	4.83±0.166*	5.66
5	Vimal mustard oil	8.8±0.152***	13.6
6	Olive oil	3.66±0.166	3.32
7	Parachute coconut oil	3.53±0.202	3.06
8	Palm oil	8.56±0.233	13.12
9	Amul butter	7.6±0.208***	11.2
10	Amul ghee	5.6±0.264	7.2
11	Vanaspati ghee	6.5±0.288	9
12	Vanaspati dalda	9.5±0.288***	15

***P<0.001, **P<0.01, *P<0.05.

oxidation level and the greater the peroxide value, the more oxidized the oil is. Table 5 showed that Nutrela refined soya bean oil has significantly high peroxide value (19.12 meg peroxide/kg) and hence high degree of unsaturation. This observation helps to suggest that Nutrela refined soya bean oil has high content of unsaturated fatty acids, linoleic and oleic acid which is responsible for oxidative rancidity.

Discussion

The experiment was performed with an objective of quantification of cholesterol level in different edible oils available in local market of Mizoram by using a simple Liebermann-Burchard method. All the samples were found to contain cholesterol in a closely related amount. Daily requirements of cholesterol are estimated

to be approximately 300 mg per day and thus showing the tested samples to be within the safety level.

Mizoram cuisine constitutes use of cooking oil as a prime ingredient either in household or in business. However, with the uprising health problems related to cholesterol, it is essential to have a clear clarification on the edible oils consumed as to know their safety towards the related illness. On which, one can choose or decide a safe route for his health and thus practice necessary precautions in order to avoid certain outcomes.

In order to maintain a body from possible ill-problems related to cholesterol, the following assumptions may be considered. First and simplest way to reduce cholesterol in the blood is to eat foods containing low cholesterol. Second, reducing the total calorie intake and by decreasing the amount of ordinary fat in the diet which usually causes reduction of the blood cholesterol concentration. Third, controlling the amount and type of fats consumed in the diet without altering calorie intake by choosing polysaturated/vegetable oils instead of saturated fats/ oils.[10]

References

1. Hongbao, M., & Shieh, K.J. (2006). Cholesterol and human health. *The Journal of American Science* **2**, 46-50.
2. Okpuzor, J., Okochi, V.I., Ogbunugafor, H.A., Ogbonnia, S., Fagbayi, T. & Obidiegwu (2009). Estimation of cholesterol level in different brands of vegetable oils. *Pakistan Journal of Nutrition* **8**, 57-62.
3. The Physicians Committee. Cholesterol and Heart Disease. www.pcrm.org/health/health-topics/cholesterolandheartdisease (16 September 2017)
4. Gashaw, A. & Getachew, T. (2014). Cholesterol content and free fatty acids in edible oils and health effects: A review. *International Journal for Research in Applied Science and Engineering Technology*, 120-125.
5. Mehta, S.L., Lotha, M. & Sane, P.V. (1983). Recent advances in the plant Biochemistry, *Indian Council of Agriculture Research New Delhi*, pp 14-20.
6. Sharma, B.K. (2000). Cholesterol in health and disease. The Sunday Tribune-Spectrum-Fitness.htm.
7. Daksha, A., Jaywant, P., Bhagyashree, C. & Subodh, P. (2010). Estimation of sterols contents in edible oils and ghee samples. *International Journal of Pharmceutical Sciences Review and Research* **5**, 135-137.
8. Pearson, D. (1981). *The Chemical Analysis of Food*. Churchill Publishing, London, pp 580-581.
9. AOCS (2003). Official Method Cd 8-53. American Oil Chemists Society, Campaign, IL. www.bioriginal.com (12 August 2017).
10. Sabir, S.M., Hayat, I. & Gardezi, S.D.A. (2003). Estimation of sterols in edible fats and oils. *Pakistan Journal of Nutrition* **2**, 178-181.

Sci Vis **18 (2)**, 70—73 (2018)

SCIENCE VISION

Available at
www.sciencevision.org

Research Article

OPEN ACCESS

Effect of seasonal variation on soil enzymes activity and fertility of soil in paddy fields of North Vanlaiphai, Mizoram, India

C. Vanlalveni, R. Lalfakzuala*

Laboratory of Microbiology, Department of Botany, Mizoram University, Aizawl 796004, India

For sustainability in agricultural productions, familiarity of soil quality and manual improvement to create the best possible growing environment for plants are extremely important. In this study the quality of soil of a paddy field in North Vanlaiphai was investigated with respect to the change in seasons throughout the year. A total of five (5) soil samples were selected from various places of the paddy field, and soil fertility indicators such as pH, total soil nitrogen (N), available phosphorus (Pav), exchangeable potassium (Kex), soil organic carbon (SOC), soil organic matter (SOM) and soil enzymes *viz.* dehydrogenase, phosphatase and urease were analyzed using standard protocols. All the parameters except available phosphorus and exchangeable potassium were found to be highest during rainy season whereas lowest in summer.

Key words: Soil enzymes, physico-chemical properties, soil fertility.

Received 07 March 2018
Accepted 16 April 2018

*For correspondence ✉:
lalfaka@yahoo.com

Contact us ✉:
sciencevision@outlook.com

Introduction

In context of sustainable agricultural production, soil testing is very important as it provides the conditions of available nutrients which indicates the fertility and productivity of the soils.[1] Nitrogen, phosphorus, potassium are important elements that dictate its fertility and yields of the crops.[2] Agricultural lands are multifunctional, providing a range of regulating, supporting and cultural ecosystem services in addition to food, fodder, fuel and fibre. This 'underscores the need to manage agricultural areas as multifunctional systems... not as ecological sacrifice zones'.[3]

Soil fertility is the capacity of a soil to supply essential plant nutrients in adequate amounts to facilitate optimum growth and production of a crop. Typically, inorganic nutrients such as N, P and C are usually found at high concentration due to the anthropogenic influence (e.g. fertilization, wastewater, agricultural procedures).[4] It was observed that the nutrient concentration changes along the crop cycle, very much related to the agricultural techniques used in the ricefields as well as with the sediment status. Soil fertility and quality play a pivotal role in achieving the promising yield of the crops. However, unless properly managed, soil fertility as well as quality decline drastically with intensive farm-

ing. Maintenance of soil fertility is therefore very important for sustaining high yields of vegetation. In this line, a soil test can be an important management tool in developing an efficient soil fertility program and monitoring a field for potential soil and water management problems. A soil test provides basic information on the nutrient supplying capacity of the soil. The objective of this paper was to analyze the trend in fertility status of a paddy field of North Vanlaiphai, Mizoram with respect to seasonal variations.

Soil enzymes increase the reaction rate at which plant residues decompose and release plant available nutrients. The enzymatic reaction releases a product, which can be a nutrient contained in the substrate. Sources of soil enzymes include living and dead microbes, plant roots and residues, and soil animals. Enzymes stabilized in the soil matrix accumulate or form complexes with organic matter (humus), clay, and humus-clay complexes, but are no longer associated with viable cells. Enzymes respond to soil management changes long before other soil quality indicator changes are detectable. Soil enzymes play an important role inorganic matter decomposition and nutrient cycling. Some enzymes only facilitate the breakdown of organic matter (e.g., hydrolase, glucosidase), while others are involved in nutrient mineralization (e.g., amidase, urease, phosphatase, sulfates). The relationship may be indirect considering nutrient mineralization to plant available forms is accomplished with the contribution of enzyme activity.

Methodology

Analysis of soil physico-chemical parameters

Soil pH, bulk density, water holding capacity and soil moisture content was determined by using the method of Bashour and Sayegh[5]. Soil organic carbon, total soil nitrogen, available phosphorus was estimated by the method described by Walkley and Black[6], Jackson *et al.*[7] and Olsen *et al.*[8] respectively. Soil enzymes, dehydrogenase, Phosphatase and urease were determined by using the method of Casida *et al.*[9] Tabatabai *et al.*[10] and Mc Garity *et al.*[11], respectively.

Statistical analysis

All data are presented as means of three replicates with standard error. Differences between variables were tested with standard one-way analysis of variance (ANOVA), significant differences existed in all the parameters except parameter 3 that is Bulk density (**Table 4**). Differences were considered as significant at $P < 0.05$ levels. The statistical analyses were performed using SPSS software (Standard release version 16 for windows, SPSS Inc., IL, USA).

Results and Discussion

Soil parameters were studied in different seasons of the year *viz.* rainy season, winter and summer. Results showed that all the soil parameters varied under the influence of seasonal variations (**Table 1 & 2**). The pH of the soil found to be lowest in rainy season (4.7), followed by winter and highest in summer (5.29). Decreased pH during rainy season may be due to decomposition of organic matter which releases organic acids leading to leaching of bases under prevailing high rainfall.[12-13] Soil organic carbon; soil organic matter and total soil nitrogen were highest during rainy season. During this study period, amount of nitrogen in kg ha^{-1} followed the order rainy season (0.27)>winter (0.17)>summer (0.14). When soil is warm and moist, decomposition proceeds rapidly and nitrogen released from

Table 1 | Soil physical properties.

Soil properties	Rainy season	Winter	Summer
Temperature (˚C)	25.27 ± 0.08	19.33 ± 0.23	25.97 ± 0.22
Moisture Content (%)	77.8 ± 2.90	46.65 ± 1.81	8.03 ± 0.11
Bulk Density (gm/cm^3)	0.91 ± 0.02	1.05 ± 0.02	1.03 ± 0.03

Table 2 | Soil chemical properties.

Soil properties	Rainy season	Winter	Summer
Soil pH	4.7 ± 0.01	4.87 ± 0.07	5.29 ± 0.00
Soil organic carbon (%)	2.86 ± 0.04	1.34 ± 0.00	1.02 ± 0.03
Soil organic matter (%)	4.93 ± 0.08	2.31 ± 0.00	1.76 ± 0.06
Total soil nitrogen (%)	0.27 ± 0.00	0.17 ± 0.00	0.14 ± 0.00
Available phosphorus (kg/ha)	3.96 ± 0.04	4.23 ± 0.02	3.64 ± 0.05
Exchangeable potassium (kg/ha)	136.38 ± 3.05	135.43 ± 1.24	151.3 ± 1.11

Table 3 | Soil enzyme activity.

Enzymes	Rainy season	Winter	Summer
Dehydrogenase (µg TPF/gm dry soil/24hrs)	0.81 ± 0.06	0.13 ± 0.00	0.09 ± 0.00
Phosphatase (µg p-NPP/gm dry soil/hr)	89.86 ± 1.70	49.36 ± 1.34	48.73 ± 0.78
Urease ($NH4^+$-N/ml/3hrs)	1.10 ± 0.01	0.84 ± 0.00	0.56 ± 0.00

Table 4 | One-way analysis of variance (ANOVA).

Sl. No.	Parameters	Source of variance	f-value	p-value
1	Soil temperature	Rainy season X Winter X Summer	178.4229*	.000005*
2	Soil moisture content	-do-	156.0891*	.000007*
3	Bulk density	-do-	3.5191	.097453
4	Soil pH	-do-	21.9284*	.001743*
5	Soil organic carbon	-do-	382.6270*	.000000*
6	Soil organic matter	-do-	388.1868*	.000000*
7	Total soil nitrogen	-do-	259.4000*	.000001*
8	Available phosphorus	-do-	27.4648*	.000955*
9	Exchangeable Potassium	-do-	9.7898*	.012905*
10	Dehydrogenase	-do-	125.4613*	.000013*
11	Phosphatase	-do-	227.8959*	.000002*
12	Urease	-do-	533.4444*	.000000*

crop residues may be significant, but when soil is cold or very dry, nitrogen released may be lesser.[14]

Percentage of soil organic carbon and soil organic matter followed the order rainy season (2.86 and 4.93)>winter (1.34 and 2.31)>(1.02 and 1.76), respectively. Soil carbon content is positively correlated with soil organic matter.[15] Low level of phosphorus and potassium may be attributed to removal of crop residues and grazing of livestock,[16] leaching from poor sandy soil,[17] and due to high rainfall.[18]

Soil enzymes showed the same trend in our data (Table 3), highest in rainy season and lowest during summer. Soil enzymes have varying optimum pH and temperature at which they function most efficiently. Their structure and substrate binding ability can be altered by heat and extreme cold temperature. The activity of many soil enzymes often correlates with soil moisture content, drought may suppress enzyme activity. Chhonkar *et al.*[19] described positive correlation of phosphates activity with soil organic carbon and negative correlation with soil pH. Soil enzymes activity can be related to soil organic matter and total soil nitrogen.[20-21] All these features could be attributed to the increased amount of enzyme activity during rainy

season and decreased rate during summer.

Conclusion

The comparative study of biochemical and physico-chemical properties of N. Vanlaiphai paddy field soil during the three seasons' viz. rainy, winter and summer, showed significance ($p<0.05$) variation among all the parameters except bulk density. It t is assumed that different seasonal pattern has effect on biochemical and physico-chemical properties of the soil.

Acknowledgement

The authors are thankful to Council of Scientific and Industrial Research (CSIR), New Delhi, Government of India for financial assistance.

References

1. Sahrawat, K.L. & Wani, S.P. (2013). Soil testing as a tool for on-farm fertility management: experience from the semi-arid zone of India. *Communications* in *Soil Science* and *Plant Analysis* **44**, 1011-1032.
2. Tomer, M.D. & Liebman, M. (2014). Nutrients in soil water under three rotational cropping systems Iowa, USA. *Agriculture Ecosystem and Environment* **186**, 105-114.
3. Milder, J.C., Garbach, K., DeClerck, F.A.J., Driscoll, L. & Montenegro, M. (2012). An assessment of the multifunctionality of agroecological intensification. A report prepared for the Bill & Melinda Gates Foundation.
4. Penüelas, J., Poulter, B., Sardans, J., Ciais, P., van der Velde, M., Bopp, L., Boucher, O., Godderis, Y., Hinsinger, P., Llusia, J., Nardin, E., Vicca, S., Obersteiner, M. & Janssens, IA. (2013). Human-induced nitrogen-phosphorus imbalances alter natural and managed ecosystems across the globe. *Nature Communication* **4**, 1-10.
5. Bashour, II. & Sayegh, A.H. (2007). Methods of analysis for soils of arid and semi-arid regions. *Food and Agriculture Organization of the United Nations*. Rome 15-37.
6. Walkley, A. & Black, I.A. (1934). An examination of the Degtjareff method for determining organic carbon in soil: In effect of variation in digestion condition and of inorganic soil constituent. *Soil Science* **63**, 251-263.
7. Jackson, M.L. (1973). *Soil Chemical Analysis*. New Delhi, Prentice Hall India (P) Limited.
8. Olsen, S.R., Cole, C.V., Watanabe, F.S. & Dean, L.A. (1954). Estimation of available phosphorus in soils by extraction with sodium bicarbonate. *US Department of Agriculture Circular* No. 939.
9. Casida, L.E. (1977). Microbial metabolic activity in soil as measured by dehydrogenase determinations. *Applied Environmental Microbiology* **34**, 630-636.
10. Tabatabai, M.A. & Bremner, J.M. (1969). Use of p-nitrophenylphosphate for assay of soil phosphatase activity. *Soil Biology and Biochemistry* **1**, 301-307.
11. Mc Garity, J.W. & Myers, M.G. (1967). A survey of urease activity in soil of northern New South Wales. *Plant and soil* **27**, 217-238.
12. Conyers, M.K, Uren, N.C. & Helyar, K.R. (1995). Causes of changes in pH in acidic mineral soils. *Soil Biology and Biochemistry* **27**, 1383-1392.
13. Yan, F., Schubert, S. & Mengel, K. (1996). Soil pH changes during legume growth and application of plant material. *Biology and Fertility of Soils* **23**, 236-242.
14. Eckert, D. (2010) Efficient Fertilizer Use—Nitrogen. 1-19
15. Soon, Y.K. & Arshad, M.A. (1996). Effects of cropping systems on nitrogen, phosphorus and potassium forms and soil organic carbon in gray luvisol. *Biology and Fertility of Soils* **22**, 184-190.
16. Bolland, M.D.A. (1987). Phosphorus deficiency in narrow-leafed lupins. Farmnote no. 62/87, Agdex 161/632. Dept Agric. WA, Perth
17. Bolland, M.D.A., Yeates, J.S. & Clarke, M.F. (1996). Effect of fertilizer type, sampling depth, and years on Colwell soil test phosphorus for phosphorus leaching soils. *Fertilizer Research* **44**, 177-188.
18. Bolland, M.D.A., Cox, W.J. & Codling, B.J. (2002). Soil and tissue tests to predict pasture yield responses to applications of potassium fertilizer in high rainfall areas of south-western Australia. *Australian Journal of Experimental Agriculture* **42**, 149-164.
19. Chhonkar, P.K. & Tarafdar, J.C. (1984). Accumulation of phosphatases in soils. *Journal of Indian Society of Soil Science* **32**, 266-272.
20. Aon, M.A. & Colaneri, A.C. (2001). Temporal and spatial evolution of enzymatic activities and physico-chemical properties in an agricultural soil. *Applied Soil Ecology* **18**, 255-270.
21. Noorbakhsh, F., Hajrasuliha, S. & Emtiazy, G. (2001). Factors affecting urease enzyme activity in some soils in Isfahan Province. *JWSS-Isfahan University of Technology* **5**, 95-106.

Sci Vis **18 (2)**, 74—77 (2018)

SCIENCE VISION

Available at
www.sciencevision.org

Research Note

OPEN ACCESS

Estimation of current population mean using two-occasion successive sampling with one auxiliary variable

R. Zoramthanga

Department of Statistics, Pachhunga University College, Aizawl 796001, India

In this study, two-occasion successive sampling for ratio-to-regression estimator was used to determine the current estimate of the population mean using only the matched part and one auxiliary variable, which is available on both the occasions. The data used were based on the total number of female workers in villages in Mizoram with the total number of literate female in villages in Mizoram as an auxiliary variables. The data were gotten from Census of India 2001 and 2011. The optimum mean square error of the combined ratio-to-regression and ratio estimator has been compared with (i) the optimum mean square error of the chain-type ratio estimator (ii) mean per unit estimator and (iii) combined estimator when no auxiliary information is used at any occasion. This result showed that the combined ratio-to-regression and ratio estimator is more efficient than the other three existing estimators.

Key words: Ratio-to-regression estimator, two-occasion successive sampling, mean square error (MSE), relative efficiency.

Received 27 February 2018
Accepted 12 April 2018

*For correspondence ✉:
aramaralte7@gmail.com

Contact us ✉:
sciencevision@outlook.com

Introduction

In a sample survey, it is often seen that sample surveys are not limited to one time enquiries. If the value of study character of a finite population is subject to change over time, a survey carried out on a single occasion will provide information about the characteristics of the surveyed population on the given occasion only and cannot give any information on the nature or the rate of change of the characteristics over all the occasions or more recent occasion. Data regarding changing properties of the population of cities or countries, such as unemployment statistics, are collected regularly on a sample basis, to estimate the change from one occasion to the next or to estimate the average over certain period. An important aspect of continuous survey is the structure of the sample on each occasion. To meet these requirements, successive sampling provides a strong tool for generating reliable estimates on different occasions. When the same population is sampled repeatedly the sampler is in an ideal position to make realistic estimates, both of costs and of variances and to apply the technique that leads to optimum efficiency of sampling.[1] The successive sampling is a known technique that can be used in longitudinal survey to estimate the population parameters and measurements of difference or change of a study variable.

Successive sampling is used extensively in

applied sciences, social sciences and economic research. There are several types of procedures to adopt for estimating the population parameters:

The same sample may be used on each occasion.

A new sample may be taken on each occasion.

A part of the sample may be retained while the remainder of the sample may be drawn afresh.

A sub sample of the original sample on the second occasion.[2]

Generally, the main objective of successive surveys is to estimate the change with a view to study the effects of the forces acting on the population.[3]

Here some conditions to consider are that;

For estimating change from one occasion to the next, it may be best to retain the same sample on each occasion

For estimating the mean on each occasion, it may be best to draw a fresh sample on each occasion, and

If it is desired to estimate the mean on each occasion and also the change from one occasion to the next, it may be best to retain part of the sample and draw the remainder of the sample afresh.

A large part of sample survey theory has been directly motivated by practical problems encountered in the design and analysis of large scale sample surveys. Major advances have taken place in handling both sampling and non-sampling errors as well as data collection and processing. Since then considerable amount of work has been done in the direction of providing improved estimators in survey sampling. The sampling on two-occasion was first considered by Jessen[4] for the estimation of current population mean. Patterson[2] studied a method of partial matching to estimate the mean on each occasion and also the change from one occasion to the next.

Rao and Graham,[5] Gupta,[6] and Sen[7] developed estimators for the population mean on the current occasion using information on two auxiliary variables available on the previous occasion. Sen[8,9] extended his work for several auxiliary variates. Singh and Talwar[10] and Singh and Singh[11] used the auxiliary information on current occasion for estimating the current population mean in two occasion successive sampling.

Singh and Priyanka[12-14] proposed varieties of chain-type ratio, difference and regression estimators for estimating the population mean at current (second) occasion in two occasion successive sampling.

Eze *et al.*[15] studied successive sampling for regression estimation to determine the current estimate of the population mean, minimum variance, maximum precision, estimate of change between the two successive occasions under consideration and estimate of average over the period of two occasions. Also the used of successive sampling to determine the current estimate of the mean, minimum variance, estimate of change between the two successive occasions and estimate of average over the period of the two occasions was studied.[16]

The intention of this paper is therefore to find out if the combined ratio-to-regression and ratio estimator [17] has a greater efficiency than the chain-type ratio estimator,[18] mean per unit estimator and combined estimator when no auxiliary information is used at any occasion suggested by Cochran.[1]

Materials and Method

Data used

The data used for this study is from the records of the total number of female workers and the total number of literate female in villages in the state of Mizoram, Census of India 2001 and 2011.[19]

Methodology

The variables x(y) were defined as the total number of female workers in villages in the state of Mizoram, India in 2001 (2011) and z is defined as an auxiliary variable which is the total number of literate female in villages in the

state of Mizoram, India.

Consider a population consisting of N units. Let a character under study on the first (second) occasion be denoted by x(y), respectively. It is assumed that the information on an auxiliary variable z is available on the first as well as on the second occasion. It is also assumed that the population to be large enough, and the sample size is constant on each occasion. Using simple random sampling without replacement (SRSWOR) select a sample of size n on the first occasion. Of these n units, a sub-sample of size $m=n\lambda$ is retained on the second occasion. This sub-sample is supplemented by selecting an SRSWOR of $u=(n-m) = n\mu$ units afresh from the units that were not selected on the first occasion.

Results

From the data used, using two occasion successive sampling a random sample of 70 villages was selected from a population of 669 villages on each occasion, this comprises of 35 matched villages and 35 unmatched villages in the state of Mizoram.

Table 1 | Relative Efficiency (%) of T_{p_1} with respect to estimators T, $\bar{y}_n$ and $\bar{y}_2'$.

Estimators	Estimates	MSE	Efficiency %
T_{p_1}	146	107.49	100
T	139	344.65	320.62
$\bar{y}_n$	130	373.54	347.50
$\bar{y}_2'$	130	304.81	283.56

where

T_{p_1} is a combined estimator proposed by Ralte and Das,[17]

T is a combined estimator proposed by Singh,[18]

$\bar{y}_n$ is mean per unit estimator, and

$\bar{y}_2'$ is a combined estimator suggested by Cochran[1] when no auxiliary information is used at any occasion.

Conclusion

From the table in the above section, the combined ratio-to-regression and ratio estimator i.e. T_{p_1} is more efficient than the other existing three estimators viz. T, $\bar{y}_n$ and $\bar{y}_2'$ with maximum gain in efficiency occurring while comparing with mean per unit estimator, which is very obvious. Hence, the estimator T_{p_1} is recommended for further practical use.

References

1. Cochran, W. G. (1977). *Sampling Techniques* (3rd ed.). Wiley Eastern Ltd.
2. Patterson, H. D. (1950). Sampling on successive occasions with partial replacement of units. *Journal of the Royal Statistical Society* **12**(B), 241 – 255.
3. Singh, D. & Chaudhary, F.S. (1986). *Theory and Analysis of Sample Survey Designs* (1st ed.). New Age International (P) Ltd.
4. Jessen, R.J. (1942): Statistical investigations of a sample survey for obtaining farm facts. *Journal of Agricultural Experiment Station Research Bulletin* **304**, 1–104.
5. Rao, J.N.K. & Graham, Jack E. (1964). Rotation designs for sampling on repeated occasions. *Journal of American Statistical Association* 59, 492–509.
6. Gupta, P.C. (1979). Sampling on two successive occasions. *Journal of Statistical Research* 13, 7-16.
7. Sen, A.R. (1971). Successive sampling with two auxiliary variables. *Sankhya* **33**(B), 371–378.
8. Sen, A.R. (1972): Successive sampling with p (p≥1) auxiliary variables. *The Annals of Mathematical Statistics* **43**, 2031–2034.
9. Sen, A.R. (1973). Theory and application of sampling on repeated occasions with several auxiliary variables. *Biometrics* **29**, 381–385.
10. Singh, P. & Talwar, H.K. (1991). Estimation of population regression coefficient in successive sampling. *Biometrical Journal* **33**, 599–605.
11. Singh, G.N. & Singh, V.K. (2001). On the use of auxiliary information in successive sampling. *Journal of the Indian Society of Agricultural Statistics* **54**(1), 1–12.

12. Singh, G.N. & Priyanka, K. (2006). On the use of chain-type ratio to difference estimator in successive sampling. *International Journal of Applied Mathematics and Statistics* **5**(S06), 41–49.
13. Singh, G.N. & Priyanka, K. (2007). On the use of auxiliary information in search of good rotation patterns on successive occasions. *Bulletin of Statistics and Economics* **1**(A07), 42–60.
14. Singh, G.N. & Priyanka, K. (2008). On the use of several auxiliary variates to improve the precision of estimates at current occasion. *Journal of the Indian Society of Agricultural Statistics* **62**(3), 253–265.
15. Eze, S.O., Amahia, G.N., Olayiwola, O.M. & Adewara, A.A. (2011). Efficiency of second occasion over first occasion on successive sampling for regression estimation. *Journal Mathematics Research* **3**(2), 235–242.
16. Anieting, A.E. & Ezugwa, V.O. (2013). Estimation of the current occasion parameters using successive sampling approach. *American Journal of Engineering Research* **2** (9), 204–207.
17. Ralte, Z. & Das, G. (2015). Ratio-to-regression estimator in successive sampling using one auxiliary variable. *Statistics in Transition* **16**(2), 183–202.
18. Singh, G.N. (2005). On the use of chain-type ratio estimator in successive sampling. *Statistics in Transition* **7** (1), 21–26.
19. Census of India 2001 and 2011. www.cesusindiagov.in

Sci Vis 18 (2), 78—80 (2018)

SCIENCE VISION

Available at
www.sciencevision.org

Research Note

OPEN ACCESS

Sightings of chestnut-flanked white-eye *Zosterops erythropleurus*: First report from Mizoram, India

Lalawmawia Sailo[1*], Lawmsangzuala Chhangte[2], Rochamliana Aineh,[3] H. Lalthanzara[4]

[1]Department of Zoology, Mizoram University, Aizawl 796004, India
[2]Khatla, [3]Chanmari, Aizawl 796001, India
[4]Department of Zoology, Pachhunga University College, Aizawl 796001, India

In this paper we report the first confirmed sighting of chestnut-flanked white-eye *Zosterops erythropleurus* (Swinhoe, 1863) from Mizoram and first photographic report from India. chestnut-flanked white-eye belongs to the family Zosteropidae and is a resident of Cambodia, China, Hongkong, Korea, Democratic People's Republic of Korea, Laos, Myanmar, Russia, Thailand and Vietnam. In the Indian Sub-continent this is the only sighting report for this species and details of sightings and habitat are discussed.

Key words: Chestnut-flanked white eye, Mizoram, India, first report.

Received 15 March 2018
Accepted 21 April 2018

*For correspondence ✉:
victorsailo87@gmail.com

Contact us ✉:
sciencevision@outlook.com

Chestnut-flanked white-eye *Zosterops erythropleurus* (Swinhoe, 1863) belongs to the family Zosteropidae. This diverse old world passerine family constitute a group of small, gregarious, arboreal birds having a wide range of distribution occupying tropical, sub-tropical and temperate Sub-Saharan Africa, southern and eastern Asia, Australasia and the tropical islands of the Indian Ocean, the western Pacific Ocean, and the Gulf of Guinea region.[1] Although they occupy wide range of geographical area, morphological variation within the family is negligible, leading most members to be homogenous in appearance. The member of Zosteropidae exhibits remarkable uniformity in their morphology with greenish-olive above and pale grey below.

As their common name suggest, many species have a conspicuous white ring of tiny white feathers around the eyes. The size of this white eye-ring may differ between species, being highly conspicuous in some taxa and reduced or absent in other species. Some species have a bright yellow throat, white or cream coloured breast and several have buff flanks.[1] All species of the family Zosteropidae are highly sociable and form large flocks that separate on the onset of the breedingseason.[2] Species belonging to the family Zosteropidae are very vocal, but tend to have weak rather simple vocalizations that can be carried far. Zosteropidae are mainly insectivorous, but can be generalist eating nectar and fruits of various kinds.[2]

Chestnut-flanked white-eye *Z. erythropleurus* is a resident of Cambodia, China, Hongkong, Korea, Democratic People's Republic of Korea, Laos, Myanmar, Russia, Thailand and Vietnam.[3] Otgonbayar *et al.*[4] also reported the first sighting of *Z. erythropleurus* from Mongolia.

This report put forward and confirmed the presence of chestnut-flanked white-eye *Z.*

Figure 1 | Chestnut-flanked white eye *Zosterops erythropleurus* at Chaltlang, Aizawl.

Figure 2 | Chestnut-flanked white eye *Z. erythropleurus* at Murlen National Park.

erythropleurus in Mizoram and also the first photographic record of the species from the Indian sub-continent. The chestnut-flanked white-eye *Z. erythropleurus* was first sighted on the 6 February 2015, 0856 hrs at a cliff top mixed vegetation of bamboo and small trees inside the area of Chaltlang locality of Aizawl city. The mix flock comprise of two *Z. erythropleurus* and six *Z. palpebrosus*. The encounter site is at an elevation of 1103.07 m above sea level with geo-co-ordinate 23°45'09.13"N 92°43'20.40"E. The sighting was not supported by a high quality photograph which leaves the authors puzzled with the confirmation of the species. The photograph (**Fig. 1**) from the first sighting was inferior in quality due to the poor visibility with a thick fog. Two individuals of *Z. erythropleurus* were encountered among the flock of Oriental white-eye *Z. palpebrosus*, a commoner in the area.

The second and third author re-confirmed the species *Z. erythropleurus* with photograph from Murlen National Park located on the eastern side of Mizoram on the 16 and 18 January 2018. At around 1015 hrs in a bright morning, five *Z. erythropleurus* were seen among the flock of 40 *Z. palpebrosus* (**Fig. 2**) feeding on the nectar of *Leucosceptrum canum* (local name: *kawihṭhuang*) at 23°38'54.19"N 93°17'28.80"E at an elevation of 1640.73 m.

The sightings from the state of Mizoram are only during the winter months which suggest that the birds are migrating from their home range and are readily forming a mixed flock with the closely related resident species of the family.

Acknowledgement

The authors convey their heartiest gratitude to the Range Officer and Staffs of Murlen National Park and to all those who extend their help during the study.

References

1. Van Balen, B. (2008). Family Zosteropidae (white eye). Pp. 402-485 in J. del Hoyo, A., Elliott, J., and Christie, D. A., eds, (2008). *Handbook of the Birds of the World*, 13, Barcelona: Lynx Editions.
2. Moreau, R.E. (1957) Variation in the western Zosteropidae (Aves). *Bulletin of the British Museum,* **4**, 318–433.
3. BirdLife International. (2016). *Zosterops erythropleurus*. The IUCN Red List of Threatened Species 2016: e.T22714027A94398060. http://dx.doi.org/10.2305/IUCN.UK.2016-3.RLTS.T22714027A94398060.en. Downloaded on 25 January 2018.
4. Otgonbayar, B., Suuri, B., & Reading, R.P. (2013). Chestnut-flanked White-eye *Zosteropserythropleurus*: First record for Mongolia. *Birding Asia* **20**, 111–112.

Sci Vis **18 (2)**, 81-82 (2018)

SCIENCE VISION

Available at
www.sciencevision.org

21st MAS General Body Meeting cum seminar on Science and Technology for a Sustainable Future

21st MAS General Body Meeting cum seminar on Science and Technology for a Sustainable Future was on 30 April 2018 at the Seminar Hall, Pachhunga University College, Aizawl, Mizoram. The seminar was funded by the National Council for Science & Technology Communication, Department of Science and Technology, New Delhi, through Directorate of Science and Technology, Government of Mizoram. Keynote address was delivered by Dr R. K. Lallianthanga, MAS Chief Patron and Chief Scientific Officer, DST. Technical presentation was made by Dr Aldrin Malsawmtluanga, MAS Governing Body member and Scientific Officer, DST. Thesis presentations from new PhD holders were a bonus.

The General Body meeting included election of new Office Bearers for 2018-2010 term as follows:

President	Dr	*Vice-President*	Dr H. Lalthanzara
Secretary	Dr Lalrokima Chenkual	*Asst. Secretary*	Dr K. Lalchhandama
Treasurer	Dr Esther Lalhmingliani	*Finance Secretary*	Dr John Zothanzama

MAS OBs and Chief Patron (L-R) | H. Lalruatsanga, Lalrokima Chenkual, Esther Lalhmingliani, R.K. Lallianthanga, K. Lalchhandama, H. Lalthanzara , John Zothanzama

ISSN (print) 0975-6175/(online) 2229-6026.

MAS Governing Body members

MAS senior Governing Body members ready to give out felicitation